

- a) obtaining a DNA sample from a fruit suspected of being infected with *Guignardia*;
 - b) providing a forward primer selected from the group consisting of SEQ ID NO: 1 and 8;
 - 5 c) providing a reverse primer selected from the group consisting of SEQ ID NO: 6 and 11; and
 - d) 10 subjecting said DNA sample and said forward and reverse primers to conditions suitable for polymerase chain reaction, amplification of said DNA in the presence of said primers indicating infection with a pathogenic *Guignardia* species.
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- 15 6. A method for differentiating pathogenic species of *Guignardia* from non-pathogenic species of *Guignardia*, comprising the steps of:
 - a) obtaining a DNA sample from a *Citrus* fruit suspected of being infected with *Guignardia*;
 - b) 20 contacting said DNA with a detectably labeled probe which selectively hybridizes with said DNA, said probe having the sequence of SEQ ID NO: said probe having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 8, AND SEQ ID NO: 9; and detecting specific hybridization if 25 any, samples demonstrating hybridization with SEQ ID NO: 1 or SEQ ID NO: 8 being indicative infection of the *Citrus* fruit with pathogenic *Guignardia* and samples demonstrating hybridization with SEQ ID NOS: 2, 3, or 9 being 30 indicative of infection of the *Citrus* fruit with non-pathogenic species of *Guignardia*.

7. A method for differentiating pathogenic species of *Guignardia* from non-pathogenic species of *Guignardia*, comprising the steps of:
 - a) obtaining a DNA sample from a *Citrus* fruit suspected of being infected with *Guignardia*;
 - b) immobilizing said DNA sample on a solid support;
 - c) probing said immobilized sample DNA with a detectably labeled probe, said probe having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 8, AND SEQ ID NO:9; and
 - d) assessing said solid support for hybridization of said probe to said immobilized DNA, samples demonstrating hybridization with SEQ ID NO: 1 or SEQ ID NO: 8 being indicative infection of the *Citrus* fruit with pathogenic *Guignardia* and samples demonstrating hybridization with SEQ ID NOS: 2, 3, or 9 being indicative of infection of the *Citrus* fruit with non-pathogenic species of *Guignardia*.
8. A method of screening for *Citrus* Black Spot disease, said method comprising
 - a) obtaining a nucleic acid sample; and
 - b) assaying the nucleic acid sample for the presence of a sequence which hybridizes with SEQ ID NO: 1 or SEQ ID NO: 8 or SEQ ID NO: 4, wherein the presence of said hybridizing sequence is indicative of pathogenic *Guignardia* infection causing *Citrus* Black Spot disease.
9. A method in accordance with claim 5, wherein said assaying further comprises amplification

of the ITS region of *Guignardia* rDNA containing an ITS nucleic acid, said ITS nucleic acid sequence being amplifiable using primers consisting of SEQ ID NO: 12 and SEQ ID NO: 13.

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10. A method in accordance with claim 5, wherein said polymerase chain reaction is primed by SEQ ID NOS: 8 and 11.

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11. A kit for use in screening for *Citrus Black Spot* disease comprising a pathogen-specific oligonucleotide probe immobilized on solid matrix, and further comprising means for amplifying a test samples's nucleic acid encoding all or part of the rDNA gene, wherein said test sample's nucleic acid comprises a sequence of SEQ ID NO: 4.

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12. A kit in accordance with claim 11, wherein said amplifying means comprises:

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- (a) a primer pair of oligonucleotides comprising a first oligonucleotide having the sequence of SEQ ID NO: 1 or SEQ ID NO: 8 and a second oligonucleotide having the sequence of SEQ ID NO: 6 or SEQ ID NO: 11; and
- (b) reagents necessary to perform PCR.

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13. A kit in accordance with claim 12, wherein the oligonucleotide pair comprises SEQ ID NO: 8 and SEQ ID NO: 11.

14. A kit for use in screening for non-pathogenic *Guignardia* species comprising a non-pathogen-specific oligonucleotide probe immobilized on solid matrix, and further comprising means for amplifying a test samples's nucleic acid encoding all or part of the rDNA gene, wherein said nucleic acid comprises a sequence of SEQ ID NO: 5.
15. A kit in accordance with claim 14, wherein said amplifying means comprises:
 - (a) a primer pair of oligonucleotides comprising a first oligonucleotide having the sequence of SEQ ID NO: 2 or SEQ ID NO: 9 and a second oligonucleotide having the sequence of SEQ ID NO: 6 or SEQ ID NO:10 or SEQ ID NO: 11; and
 - (b) reagents necessary to perform PCR.
16. A kit in accordance with claim 15 wherein the oligonucleotide pair comprises SEQ ID NO: 9 and SEQ ID NO: 10.
17. A method for identifying pathogenic *Guignardia* species in a sample, said method comprising the steps of:
 - a) culturing *G. citricarpa*;
 - b) subjecting said cultured *G. citricarpa* to conditions which effect lysing of said *G. citricarpa*, thereby releasing DNA from said hyphae;
 - c) contacting said released DNA with a primer pair of oligonucleotides comprising a first oligonucleotide having the sequence of SEQ ID

NO: 1 or SEQ ID NO: 8 and a second
oligonucleotide having the sequence of SEQ ID
NO: 6 or SEQ ID NO: 11 under conditions where
amplification of pathogenicity-associated ITS
sequences occurs, if said pathogenic *Guignardia*
is present in said sample; and
d) detecting said amplified sequence, if
present.

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18. A method as claimed in claim 17, wherein said
amplified sequence is detected via
incorporation of a detectable label.

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19. A method as claimed in claim 17, wherein said
amplified sequence is detected by gel
electrophoresis of said amplified sample.

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20. A method as claimed in claim 17, wherein said
ITS sequences are amplified using a primer set
having SEQ ID NOS: 12 and 13 prior to
amplification of pathogenicity related
sequences employing a primer set having SEQ ID
NO: 8 and 11.

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21. A method for identification of pathogenic
Guignardia citricarpa, said method comprising:
a) contacting a tissue section containing a
black spot lesion with a permeabilization
agent;
b) contacting said permeabilized lesion DNA
with a detectably labeled oligonucleotide
having a sequence of SEQ ID NO: 1 or SEQ ID NO:
8; and

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c) detecting hybridization of said oligonucleotide to said DNA, if any, said hybridization indicating the presence of pathogenic *G. citricarpa*.